Begun in 1990, the U.S. Human Genome Project is a 13-year effort coordinated by the Department of Energy and the National Institutes of Health.

Human Genome Project video
Goals of the Project

- identify all the approximately 30,000 genes in human DNA,
- determine the sequences of the 3 billion chemical base pairs that make up human DNA,
- store this information in databases,
- improve tools for data analysis,
- transfer related technologies to the private sector, and
- address the ethical, legal, and social issues (ELSI) that may arise from the project.
Genetic Disease and Gene Therapy

- 5 to 10% of children inherit an identifiable genetic defect
- can alleviate symptoms, but cure the disease?
- many diseases all about genetics—hemophilia
Genetic Disease

- others a combination- FH- familial hypercholesterolemia- defect in LDL receptor gene- does not take up LDL and process it, so large amounts found in blood.
- rigorous diet and exercise can improve the lipid profile of someone with FH
- look at inheritance pattern and pedigree analysis
Finding a disease Gene

- Finding and cloning a disease causing gene (in the absence of all information-no probe from an animal sequence, etc.)

- How does one go about it?
Cystic Fibrosis

- CF - cystic fibrosis - most common lethal autosomal disease among Caucasians
- Abnormal glandular secretions that cause pulmonary, digestive, pancreatic and dysfunction
- Respiratory track infections and malnutrition (inability to digest food)
- One in every 1800 Caucasian children
- Rarely live past age 40 - 50% die before age 21
Some techniques used

- **Southern Blot** - DNA on gel with a DNA probe – named after discoverer (E.M. Southern)
- **Northern Blot** – RNA on gel with a DNA probe
- **Western** - proteins with antibodies
CF and Genetic Linkage

- Closer two genes are to each other on the chromosome, the more likely they are to remain together during meiosis (genetic linkage).
- So, first phase was to establish a linkage between CF and some genetic marker.
- Figures 10.2 and 10.3 - pages 210-211
- Also go over Roche Genetics exercise.
How do you know you have reached CF gene in chromosomal walk?

You look for ORF within each newly cloned sequence. Any ORF they found could be the CF gene.

Look at mRNAs of patients with CF; chose sweat glands, known to be affected in CF patients. - isolated sample from sweat glands and probed mRNA with fragment of DNA - looked for match
Proof they had CF gene

- One gene identified that is expressed differently in CF patients than in normal patients.
- Mutation found in every CF gene patients studied - not found in normal patients (looked at many patients)
- Chloride transport – deficient in secretory cells from CF patients. Cultured their cells in lab, put a normal copy of CF gene in, restored chloride ion transport - should really be CF gene.
- Technique also called positional cloning
Problems with technique

- More than one mutation can cause a defect
- Conditions need to be right to detect a single base pair change
- Salt and temperature conditions for the hybridization
- Develop a specific probe – like one in 70% CF patients
- Over 100 alleles of CF – can miss some
Genetic Diagnosis

- Determining whether or not a particular gene defect is present in an individual.
- Genetic diagnosis – adults – e.g. Huntington’s disease
- Embryonic diagnosis – in vitro fertilization; implant disease free embryos
- Prenatal Diagnosis – done before baby is born
  - Amniocentesis
  - CVS (chorionic villus sampling)
Gene Therapy to take place if (p.225)

1. Gene to be transferred is available
2. Effective method of introducing the gene into cells is necessary
3. Target tissue or cells accessible to gene therapy
4. Procedure does no harm to patient
5. Treatment significantly improves health of patient
Gene Therapy

- Transgenic humans
- Use retroviruses as vectors
- Things needed for procedure to work
  - Small inserts – need to accept non viral gene
  - Need to inactivate virus genes – no new viruses to be produced
  - Cells must be dividing – to incorporate DNA
Gene Therapy

- **Figure 10.7, p. 227** - replicative cycle of a retrovirus
- **Ex-vivo gene therapy** - **Figure 10.8, p.228**
- **Conditions needed:**
  - Take bone marrow cells out of the body
  - Insert retrovirus with gene of interest
  - Select for ones with gene
  - Put back into marrow or blood (diseases that affect the blood good targets for ex vivo gene therapy)
Problems with Gene Therapy

- Problems with ex-vivo method
  - Not enough cells get desired gene to correct problem
  - Modified cells don’t last long; need repeat treatments
• Other risks with gene therapy:
  - Cells injected may cause an immune response
  - Random insertion of retrovirus into host chromosome- may be likely in non-coding DNA, but what if it interrupts the coding DNA?
  - Too much of a good thing- too much clotting factor causing unwanted clots
Gene Therapy

Table 10.1 - diseases may benefit from gene therapy
Germ line gene therapy

- can’t deliver to a specific location in the chromosome - what would random insertion cause?? - been OK so far with transgenic animals - lots of noncoding DNA to accept gene
- few would survive genetic manipulation, fertilization, selection and implantation